PATENT
Attorney Docket No. **UCSD-07017**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Maurizio Zanetti		
Serial No.:	09/788,110	Group No.:	1642
Filed:	02/15/2001	Examiner:	Ungar, S.
Entitled:	**A Universal Vaccine And Method For Treating Cancer Employing Telomerase Reverse Transcriptase**		

DECLARATION OF MAURIZIO ZANETTI, M.D. UNDER 37 C.F.R. § 1.131

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: 11/22/2005 By: _____

Sir or Madam:

I, Maurizio Zanetti, M.D. hereby declare and state, under penalty of perjury, that:

1. I am the sole inventor of the instant U.S. Patent Application No. 09/788,110. I am a Professor of Medicine at the University of California, San Diego. My laboratory conducts research in the field of cancer immunology.

2. I have reviewed the Office Action mailed June 22, 2005, wherein the Examiner has cited as prior art a PCT application of Nadler *et al.*, WO 00/258130100059, published May 11, 2000, filed October 29, 1999 (citing a priority application dated October 29, 1998).

3. Attached are excerpts from an invention disclosure (Tab A) submitted to the University of California, San Diego, Technology Transfer and Intellectual Property Services on

October 1, 1999, including excerpts from a grant application (Exhibit 1) submitted to the State of California, Department of Health Services, Cancer Research Section on August 31, 1998. These documents are submitted as evidence that I conceived of compositions comprising at least one HLA-A2.1-restricted human telomerase reverse transcriptase peptide (hTRT) of from seven to fifteen amino acid residues in length in the United States before October 29, 1998. As also evidenced by these documents, I successfully used compositions comprising at least one HLA-A2.1-restricted hTRT of from seven to fifteen amino acid residues in length to in vitro immunize peripheral blood mononuclear cells (PBMC) of HLA-A2.1 transgenic mice (Exhibit 2), human PBMC from normal donors (Exhibit 5), and human PBMC from prostate cancer patients (Exhibit 6). Specifically, I successfully induced cytotoxic T lymphocyte (CTL) responses to the HLA-A2.1-restricted hTRT peptides p540 (SEQ ID NO: 1) and p865 (SEQ ID NO: 2) before October 29, 1999.

4. I further attest that I was diligent in working to reduce to practice the invention of compositions comprising at least one HLA-A2.1-restricted hTRT of from seven to fifteen amino acid residues, from conception before October 29, 1998, to actual reduction to practice before October 29, 1999, and constructive reduction to practice in U.S. Provisional Application No. 60/182,685, filed on February 15, 2000 (to which the instant application claims priority).

5. I further declare that all statements made herein are of my own knowledge are true, and that all statements are made on information and belief that are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application of any patent issued thereon.

Dated: 11/21/2005

Maurizio Zanetti, M.D.

PROPRIETARY INFORMATION

SD 2006-051 CASE NO.

TECHNOLOGY TRANSFER and INTELLECTUAL PROPERTY SERVICES, UCSD
INVENTION AND TECHNOLOGY DISCLOSURE FORM

A. TITLE OF INVENTION

Create a short title describing the general nature of the invention without revealing the specific details that would enable others to reproduce the invention (e.g., new anticancer compound, method for chip fabrication, etc.). Please limit the title to 60 characters.

TELOMERASE REVERSE TRANSCRIPTASE AS ANTIGEN FOR IMMUNIZATION IN CANCER

B. 1. UCSD INVENTOR(S)

List all UCSD employees or students who intellectually contributed to the invention. Please also indicate any joint or special appointment with non-UCSD institutions (e.g., VA and HHMI) in the "Position" box.

Name: MAURIZIO ZANETTI, M.D.		Position: PROFESSOR IR Joint or Non-UCSD Affiliation:
Dept.: MEDICINE Mail Code: 0368	Work Address: BONNER HALL 2430 9500 GILMAN DRIVE	
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Hm. Address:		Hm. Phone:

Name:	SS#:	Position: Joint or Non-UCSD Affiliation:
Dept.: Mail Code:	Wk. Address:	
Wk. Phone:	Fax:	Email:

ROM #1

C. DESCRIPTION OF EVENTS:

This information is important for deciding pri[illegible] of invention and/or legal "bars" to patenting. In gen[illegible] publications, in any medium, before the date a formal patent application is filed in a national patent office can cause a bar to patent filing in most foreign countries. While United States patent law allows inventors up to one year to file a patent application after the first publication, public use, or sale, the loss of foreign rights is often very important to potential industrial licensees.

EVENTS	DATE	INDICATE THE WRITTEN RECORD (e.g., notebook, letter, email). IF ORAL DISCLOSURE, INDICATE TO WHOM.
1. Initial conception of the idea	APRIL 22, 1998	CONVERSATION WITH Mr. PAULO RANGEL
2. First description of complete invention, oral or written	AUGUST 17, 1998	GRANT APPLICATION TO CRP (Cancer Research Program) State of California. - Exhibit 1.
3. First successful demonstration (first actual reduction to practice)	DECEMBER 4, 1998	EXPERIMENTAL DATA BOOK PAGE. Exhibit 2.
4. Has this work been: i. submitted for publication? (Y) N ii. accepted for publication? Y N iii. Published? Y N	SEPTEMBER 27, 1999	MANUSCRIPT SUBMITTED TO *NATURE*
5. Have you presented this work at a conference or meeting? i. Did you submit an abstract? Y (N) ii. Was abstract published? Y (N) iii Name of conference or meeting? Y (N) i. Did presentation include handouts? Y (N)		

F. INVENTORS' SIGNATURES

By signature below, I acknowledge my responsibilities and rights to royalty-sharing under the current University of California Patent Policy.

[signature] 10/1/1999

Inventor signature — Date

Inventor signature — Date

Inventor signature — Date

G. WITNESS - invention disclosed to and understood by:

[signature] 10/1/99

Witness signature — Date

Ommid Asbaghi

Print witness name

H. ABOUT THE INVENTION

1. Describe how your invention works (or may work). Please include drawings, schematics, figures, etc., necessary to explain how the invention works or may work.

To date the invention consists in the demonstration that hTRT peptides selected on the basis of their binding to the HLA molecule (Exhibit 4) can induce cytotoxic T lymphocytes in normal individuals (Exhibit 5) and in patients with cancer (Exhibit 6).

It is envisioned that immunity against hTRT can be induced using appropriately formulated hTRT synthetic peptides and genes coding for selected hTRT peptides in the form of plasmid DNA or retroviral vectors. These can be prepared according to established methods and procedures.

1. Describe the stage of development of the invention (e.g., concept stage, experimental data stage, computer model simulation stage, working prototype stage, etc.). Please include data, photographs, etc., indicating the stages of development.

The invention is presently at the proof of principle stage. Using synthetic peptides that bind in vitro to the HLA-A2.1 molecule (Exhibit 4) it has been possible to demonstrate that cytotoxic T lymphocytes can be expanded out of peripheral blood lymphocytes (circulating blood) from adult normal individuals (Exhibit 5) and patients with prostate cancer (Exhibit 6). To date two such peptides have been studied and both of them have yielded a specific immune response. Therefore, this invention is beyond the concept stage and can be rightly considered at the experimental data stage.

1. What are potential commercial applications of your invention?

It is difficult to anticipate what the commercial application might be. It is possible that peptides such as the ones studied in this invention, or other that may be studied using a similar approach, will constitute the substrate for a cancer vaccine in humans. However, due to the many obstacles, conceptual and practical, existing in the phase of development of a vaccine for human use, any prediction that hTRT peptides can be used in an appropriate form as a vaccine for humans is highly speculative.

EXHIBIT 1

CALIFORNIA CANCER RESEARCH PROGRAM - CYCLE I

APPLICATION FOR INVESTIGATOR-INITIATED, PILOT & FEASIBILITY STUDY, AND NEW INVESTIGATOR AWARDS

FACE PAGE - FORM 1A

Please follow instructions for this form

1. PROJECT TITLE (Do not exceed 60 character and spaces)
Targeted Therapeutic Vaccination in Prostate Cancer

2. APPLICATION NEW [X] REVIS [N]

3. AWARD MECHANISM	PRIORITY AREA: Primary Prevention	Epidemiology	Early Detection	Technology Transfer	Psychosocial Aspects	Innovative Treatment	General Cancer Biology	Population-Based Epidemiology TRFA	Diet/Behav. for TRFA
INVESTIGATOR INITIATED						X			
PILOT & FEASIBILITY									
NEW INVESTIGATOR									

4. PRINCIPAL INVESTIGATOR (Last, First and Middle Name) Zaretti, Maurizio — DEGREE(s) M.D.

POSITION TITLE Professor Of Medicine

COMPLETE MAILING ADDRESS (Street, Mail Stop, City, State, Zip Code) 9500 Gilman Drive, La Jolla, CA 92093-0368

DEPARTMENT, LABORATORY or EQUIVALENT Medicine — MAJOR SUBDIVISION Cancer Center

CONTACT TELEPHONE (Area Code, number, ext.) (619) 534-7217 — FAX Number (619) 822-2242 — E-mail address mzanetti@ucsd.edu

5a. HUMAN SUBJECTS [ ] No [X] Yes *If YES:* Institutional Review Board Approval Date: Pending — Institution's Assurance Of: M1274

5b. VERTEBRATE SUBJECTS [ ] No [X] Yes *If YES:* Institutional Animal Care & Use Committee Approval Date: 3/18/98, 4/15/98 — Institution's Assurance Of: A3033-01

6. DURATION OF PROPOSED PROJECT: [ ] 1 YEAR [ ] 2 YEARS [X] 3 YEARS

7. BUDGET FOR ENTIRE PROJECT PERIOD: 01-01-99 to 12-31-01 — $560,924 DIRECT COSTS — $849,797 TOTAL COSTS

8. NAME AND ADDRESS OF APPLICANT ORGANIZATION OR INDIVIDUAL
The Regents of The University of California
University of California, San Diego
9500 Gilman Dr., 0934

9. RESEARCH PERFORMANCE SITE (s) AND ADDRESS(es)
Bonner Hall
University of California, San Diego

10. CONTRACT AND GRANTS OFFICIAL:
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NAME and TITLE
Office of Contract & Grant Administration/ UCSD
ADDRESS
(619) 534-1915 TELEPHONE (Area Code, number, extension) — (619) 534-0280 FAX NUMBER — E-MAIL ADDRESS

11. OFFICIAL SIGNING FOR THE APPLICANT ORGANIZATION:
Roger D. Meyer Assoc. Dean Administration
NAME and TITLE
School Of Medicine / UCSD / 9500 Gilman Drive, 0602/ La Jolla, CA 92093
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12. PRINCIPAL INVESTIGATOR ASSURANCE: *I agree to accept responsibility for the scientific conduct and integrity of the research, and to provide the required progress reports if a grant is awarded as a result of this application.*
8/17/98
SIGNATURE OF THE PERSON NAMED IN 4. (In ink please) "PROXY" SIGNATURE NOT ACCEPTABLE

13. CERTIFICATION AND ACCEPTANCE: *I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with California Cancer Research Program terms and conditions if a grant is awarded as a result of this application.*
Roger D. Meyer
SIGNATURE OF THE PERSON NAMED IN 11. (In ink please) "PROXY" SIGNATURE NOT ACCEPTABLE

AIM 2. PEPTIDE SELECTION AND *IN VITRO* IMMUNIZATION WITH TELOMERASE PEPTIDES

Telomers form the distal ends of human chromosomes and are thought to play an important role in stabilizing the chromosomes during replication (105). Telomerase is a ribonucleoprotein, which when activated, synthesizes telomeric DNA and compensates for its loss with each cell division (106). Maintenance of a constant telomere length ensures chromosomal stability, prevents cells from aging, and confers immortality (10). An association between telomerase activity and cancer transformation of a cell has been noted. In prostate cancer 84% of prostatic carcinomas were found to be positive while only 12% of benign prostatic hyperplasia samples showed weak positivity (107). Normal tissues are free of telomerase activity (10, 107). This is true in other forms of cancer such as in 93% of breast carcinomas (108), 80% of primary lung carcinomas (109), 97% of colorectal carcinomas (110), 85% of hepatocellular carcinomas (111), and 85% of gastric carcinomas (112).

We hypothesize that human telomerase could serve as a tumor antigen in a way similar to certain oncogenes or regulators of the cell cycle, e.g., HER2/neu or p53. Consequently, peptides derived from the sequence of telomerase reverse transcriptase may be naturally expressed at surface of cancer cells in association with MHC molecules and be target of CTL based immune surveillance mechanisms. The hypothesis is corroborated by the notion that HIV-1 reverse transcriptase, an enzyme with similar functional characteristics, induces CTL responses in infected individuals (113).

The goal of this specific aim is to identify human telomerase reverse transcriptase (hTRT) peptide sequences that are immunogenic and can induce a CTL both in normal as a well as prostate cancer patients . Again central to this aspect of our work is the identification of hTRT peptides vis-à-vis which tolerance does not exist and which may may be used in the future as a component of specific vaccines.

a. Defining telomerase's antigenicity

The antigen-recognition activity of T cells is intimately linked with recognition of MHC (HLA in humans) molecules. This complex is located on chromosome 6, and encompasses nearly 200 genes encoding for MHC class I and class II among others. Because it would impossible to cover even few possibilities, the work proposed will focus on one allele only, the HLA-A2 allele, which is expressed in about 50% of the Caucasian population (114). It has been reported that about 95% of HLA-A2+ white individuals express the HLA-A2.1 subtype (115, 116). For this reason the identification of immunogenic telomerase peptides restricted by the HLA-A2.1 allele would not only serve as a proof of principle but also be applicable to 40% of the patient population with prostate cancer.

In a step-wise approach to identify those peptide sequences within hTRT (locus AF015950) (117) we based our analysis on known peptide binding motifs for the HLA-A2.1 molecule (118-120). The majority of peptides bound to MHC class I molecules have a restricted size of 9±1 amino acids and require free N- and C-terminal ends (26, 118, 121). In addition to a specific size, different class I molecules appear to require a specific combination of usually two main anchor residues within their peptide ligands (26, 118). In the case of the human allele HLA-A2.1, these anchor residues have been described as leucine (L) at position 2 and L or valine (V) at the C- terminal end (118). More recently, Ruppert et al. found that a "canonical" A2.1 motif could be defined as L or M (methionine) at position 2 and L, V, or I (isoleucine) at position 9 (120). Based on these criteria we identified 39 nine amino acid-long (9^{mer}) peptides (Figure 15).

Figure 15. Human Telomerase Reverse Transcriptase (hTRT) (From ref. (117))

1 MPRAPRCRAV RS[redacted]LPLATFVRR LGPQGWRLVQ RGDPAAFRAL VAQCLVCVPW
61 DARPPPAAPS FRQVS[redacted]LQRLCE RGAKNVLAFG FALLDGARGG PPEAFTTSVR
121 SYLPNTVTDA LRGSGAWGL[redacted]LV H[redacted]LVAPSCAYQV CGPPLYQLGA
181 ATQARPPPHA SGPRRRLGCE RAWNHSVREA GVPLGLPAPG ARRRGGSASR SLPLPKRPRR
241 GAAPEPERTP VGQGSWAHPG RTRGPSDRGF CVVSPARPAE EATSLEGALS GTRHSHPSVG
301 RQHHAGPPST SRPPRPWDTP CPPVYAETKH FLYSSGDKEQ LRPSF[redacted]
361 RETIFLGSRP WMPGTPRRLP RLPQRYWQMR PLFLELLGNH AQCPYGVLLK THCPLRAAVT
421 PAAGVCAREK PQGSVAAPEE EDTDPRRLVQ LLRQHSSPWQ VYGFVRACLR RLVPPGLWGS
481 RHNERRFLRN TKKFISLGKH AKLSLQELTW KMSVRDCAWL RRSPGVGCVP AAEHRLREEI
541 LAKFLHWLMS VYVVEL[redacted]TETTFQK N[redacted]WSKLQSIGIR QHLKRVQLRE
601 LSEAEVRQHR EARPALLTSR LRFIPKPDGL RPIVNMDYVV GARTFRREKR AERLTSRVKA
661 LFSVLNYERA RRPGLLGASV LGLDDIHRAW RTFVLRVRAQ DPPP[redacted]TGAYDTI
721 PQDRLETEVLAST IKPQNTYC VRRYAVVQKA AHGHVRKAFK SHVSTLTDLQ PYMRQFVAHL
781 QETSPLRDAV VIEQSSSLNE ASSGLFDVFL RFMCHHAVRI RGKSYVQCQG IPQGSILSTL
841 LCSLCYGDME NKLFAGIRRD GLLL[redacted]TPHLTHA KT[redacted]PEYGCVVNL
901 RKTVVNFPVE DEALGGTAFV QMPAHGLFPW CGL[redacted]QSDYSSYA RTSIRASLTF
961 NRGFKAGRNM RRKLFGVLRL KCHSLFLDLQ VNSLQTVCTN IYKILLLQAY RFHACVLQLP
1021 FHQQVWKNPT FFLRVISDTA SLCYSILKAK NAGMSLGAKG AAGPLPSEAV QWLCHQAF[redacted]
1081 [redacted]TYV PLLGSLRTAQ TQLSRKLPGT TLTALEAAAN PALPSDFKTI LD 1132

[redacted] ("L" at position 2, "L or I" at position 9)
[redacted]
("M" at position 2, "V, L or I" at position 9)

A refinement of the selection process was based on additional criteria. It has been reported that each of the non-anchor residues (position 1,3,4,5,6,7,8) has significant effect of the A2.1 binding (120). More specifically, some amino acids at position 1, 3, 6, 7, and 8 virtually abolish A2.1 binding capacity of peptides (120). Therefore, we excluded all peptides with the following amino acids at the position specified: D (aspartate) and P (proline) at position 1; K (lysine) at position 3; R (arginine) or G (glycine) at position 6; and E (glutamate) at position 7 or 8. Through this selection we excluded 12 and retained 27 peptides.

Ruppert at al. also (120) calculated the frequency of each amino acid in each of the non-anchor positions for many 9mer peptides and defined a more accurate A2.1 motif. This takes into account the impact of non-anchor positions on the A2.1 binding affinity (Table III).

TABLE III. IMPACT OF RESIDUES IN NON-ANCHOR POSITIONS ON A2.1 BINDING

PEPTIDE BINDING	POSITION	AMINO ACIDS
Good Binding Motif If:	1	Y, F, W
	3	Y, F, W
	4	S, T, C
	5	Y, F, W
No Binding If:	1	D, E, P
	3	D, E, R, K, H
	6	R, K, H
	7	D, E, R, K, H

Based on this additional step we retained 10 out of the 27 peptides (Table IV):

Table IV. TELOMERASE-DERIVED HLA-A2.1 RESTRICTED PEPTIDES

ANCHOR POSITION L at position 2 V at position 9	*ANCHOR POSITION* L at position 2 L or I at position 9	*ANCHOR POSITION* M at position 2 V, L or I at position 9
[redacted]	[redacted]	[redacted]

Whether or not the above peptides are not only HLA-binders but also CTL-inducers can only be speculated. Detailed X-ray crystallography studies have outlined the molecular structure of six different pockets in the peptide binding groove of the HLA-A2.1 molecules (122). The two main pockets (B and F) have been shown to engage the two main anchors located in position 2 and at the C-terminus of the peptide, respectively (122). The anchor residues at position 2 and the C-terminus are necessary, but not sufficient for high affinity binding, as the predictions based solely on these anchors are only about 30% accurate (123). An extended motif taking into account secondary anchor residues was reported to increase the predictability of HLA-A2.1-binding epitopes to a level of 70% (120). Therefore, we believe that approximately 7 of the 10 telomerase-derived peptides that we identified will have sufficient binding affinity to the HLA-A2.1 molecules.

b. Ascertaining CTL induction *in vitro*

We will determine whether the peptides identified as putative MHC binders can induce anti-tumor CTL responses *in vitro*. Utilizing similar approach, several class I-restricted epitopes have been identified. For example, peptides derived from the human p53 protein were able to induce primary p53-specific CTL *in vitro* (124). Tumor-reactive CTL were also induced by *in vitro* stimulation with peptides derived from the human melanoma antigens MART-1 (125) and gp100 (126), human papillomavirus epitope (127), and the HER2/neu oncogenic protein (128).

We propose to induce *in vitro* CTL specific for the telomerase-derived peptides. The *in vitro* methods are per our standard procedures. Briefly, human peripheral blood mononuclear cells (PBMC) will be isolated from HLA-A2.1+ normal volunteers (3-5 volunteers). CD8+ enriched responder cells will be plated in 24-well plates together with peptide-loaded and irradiated stimulator cells as previously described (129, 130). Cultures will be re-stimulated weekly and tested for cytolytic activity. Antigen processing-deficient T2 cells pulsed with telomerase peptides (or irrelevant 9 amino acid long peptides as control) will serve as targets. Studies will also be done to ensure that the observed cytolytic activity is in fact due to CTL and not to natural killer (NK) or lymphokine-activated killer (LAK) cells. To this end, we will use conventional target cells for NK cells (K562) or LAK cells (Daudi) to verify whether killing occurs with these targets. Moreover, we will use an antibody engineered to express three repeats of the RGD motif which blocks selectively NK and LAK cell activity but no CTL (131). As an additional specificity control we will use the anti-HLA-A2 antibody BB7.1 to block the HLA molecules at the cell surface.

c. Procedures to optimize immunogenicity of peptides

It may well be that the immunogenicity of hTRT peptides needs to be optimized. We will use two approaches, one using fusion peptides comprising signal sequences and the other making point mutations expected to increase the affinity of interaction with the MHC molecule without disrupting binding with the TCR.

1. We will design fusion peptides composed of natural or artificial signal sequences and hTRT minimal peptides. Signal sequences are those mentioned in Section C.2. We will load the fusion peptides into the cytosol of antigen processing deficient T2 cells by osmotic lysis of pinocytic vesicles. We expect that the signal sequences will translocate the minimal tumor-specific peptide from the cytosol into the ER, improving its presentation and induction of CTL. The immunogencity of fusion peptides, minimal hTRT peptides and control peptides will be compared with respect to degree of CTL activation and release of cytokines (TNF-α, IFN-γ). Since the hydrophobicity of the fusion peptides is higher than that of the minimal peptides, we will use a set of control fusion peptides with signal sequences situated on the carboxy-terminus of the minimal peptides.

2. We will modify the avidity of MHC binding by introducing amino acid substitutions at the HLA-A2.1-binding anchor positions of the hTRT peptides. Amino acid substitutions at MHC class I-binding anchor positions were shown to enhance the immunogenicity of peptides from viral antigens (132, 133) and the melanoma antigen gp100 (126). Only two - $p_{152\text{-}160}$ (LLARCALFV) and $p_{865\text{-}873}$ (RLVDDFLLV) of the 10 hTRT peptides selected (Table IV) contain L at position 2 and V at position 9 which correlate with high peptide-binding affinity (120, 134, 135). Therefore, it seems possible that analogous amino acid substitutions might enhance the ability of telomerase-derived peptides to induce telomerase-reactive CTL.

d. Induction of CTL in prostate cancer patients

Key to this proposal is to demonstrate the induction of CTL against telomerase in prostate cancer patients Success in eliciting CTL will be indication that in prostate cancer patients reactivity against hTRT has not been abrogated by tolerance.

Blood from a small group (3-5) of HLA-A2.1+ prostate cancer patients will be obtained through the courtesy of Dr. Joseph Schmidt (Department of Surgery, UCSD). The methods and procedures to induce CTL *in vitro* will be as described for PBMC from normal donors. CTL activity will be tested against T2 cells pulsed with telomerase peptides.

CTL activity will be additionally tested on prostate cancer cells. In one case we will use a panel of human prostate cancer cell lines strongly positive for telomerase activity: PC-3 (ATCC# crl1435), LNCAP (ATCC# crl1740), DU145 (ATCC# htb181), PPC1 and TSU (122). The first are identified by their ATCC catalog number and will be purchased accordingly. The latter will be requested from the investigators. HLA-

A2-negative prostate cancer cells will be used as a control. In the second case we attempt deriving cells from prostate cancer at the time of radical prostatectomy or prostatic biopsy (needle or trasurethral resection). Again patients will be HLA-typed before the surgery to identify HLA-A2.1+ patients. In all instances prior to CTL assays both cell lines and freshly isolated prostate cancer cells will be assayed for telomerase expression by PCR (123, 124). These tests will be performed in collaboration with Dr. Steven Goodison (UCSD Cancer Center).

While conducting these studies it will be important to verify whether or not CTL against hTRT also lyse normal activated peripheral blood lymphocytes since both B and T lymphocytes have been reported to undergo an increase in telomerase activity upon activation (136, 137). It should be mentioned, however, that several groups have observed that the induction of immunity against tumor antigens also expressed in normal tissues yields anti-tumor immunity but not autoimmunity (33). This applies to autoantigens expressed in lymphoid cells (138).

EXPERIMENT: 1 **DATE: 12/4/98**

TEST TELOMERASE PEPTIDES WITH CTL GENERATED *IN VITRO*

EFFECTORS: CTL63 - P540

E:T ratio	60:1	30:1	15:1	8:1	4:1	2:1
T2	2	2	3	2	1	-1
T2+P540	32	27	16	13	9	3
Jurkat	6	1	0	1	0	-1
Jurkat+P540	44	36	22	21	12	8

EFFECTORS: CTL63 - P865

E:T ratio	60:1	30:1	15:1	8:1	4:1	2:1
T2	3	4	3	2	1	-1
T2+P540	39	27	17	13	8	3
Jurkat	9	10	8	5	3	-1
Jurkat+P540	39	31	18	11	8	4

TABLE I

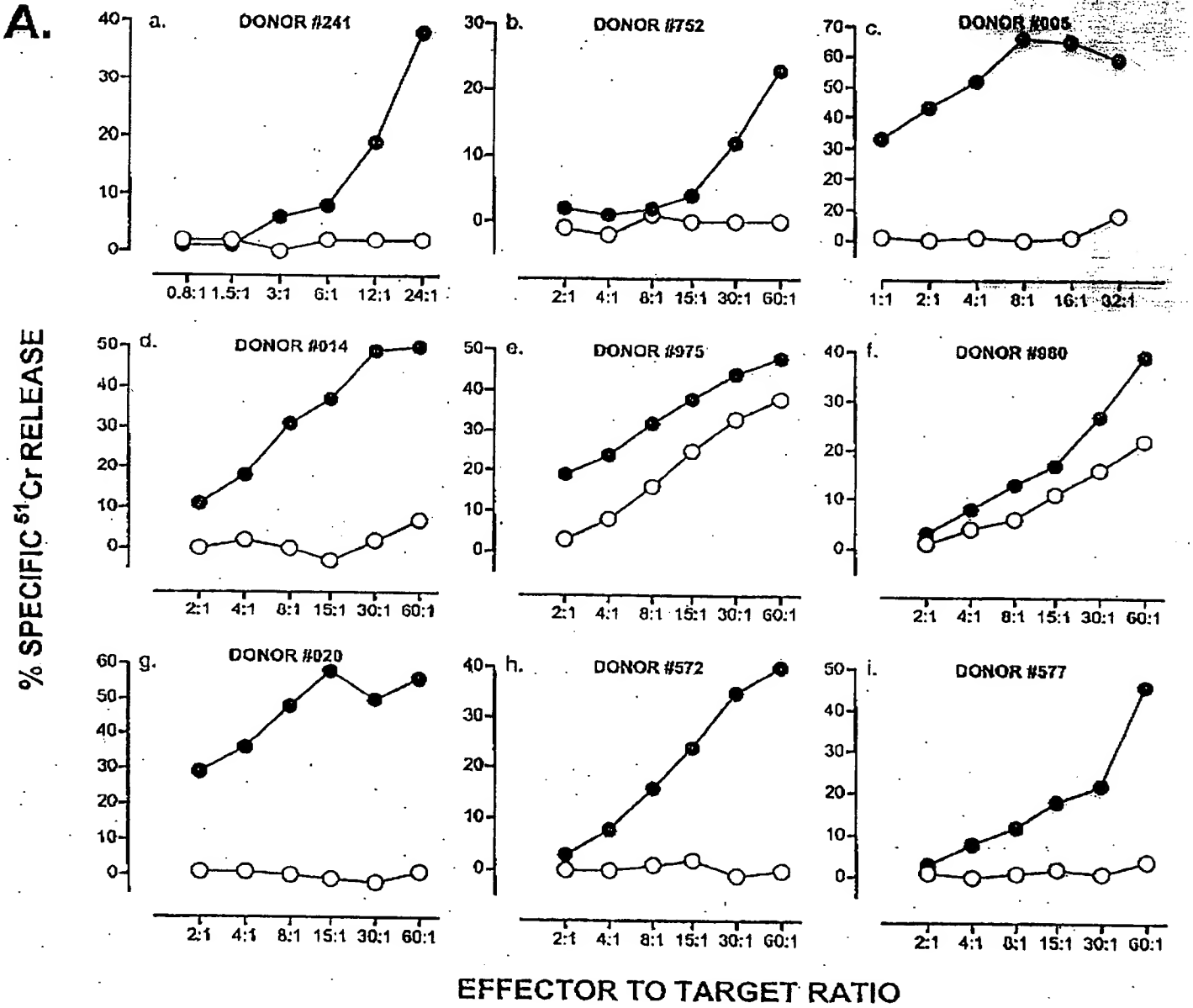
Peptide origin/ designation	Sequence	Relative Avidity (RA)[a]	DC50[b]
hTRT p540	ILAKFLHWL	2.9	4-6
hTRT p865	RLVDDFLLV	2.5	2-4
CEA p571[c]	YLSGANLNL	3	>10
gp100 p476[d]	VLYRYGSFSV	9	4-6

a. The relative avidity of hTRT peptides was measured relative to the reference peptide ILKEPVHGV at a final peptide concentration of 0.1-100 μM.

b. DC50 refers to the time required for a 50% reduction in mean fluorescence intensity.

c. Peptides of human carcinoembryonic antigen (CEA) (p571) and human melanoma antigen gp100 (p476) were used as internal controls for comparison with previously reported values [33].

EXHIBIT 5


A.
a. DONOR #241
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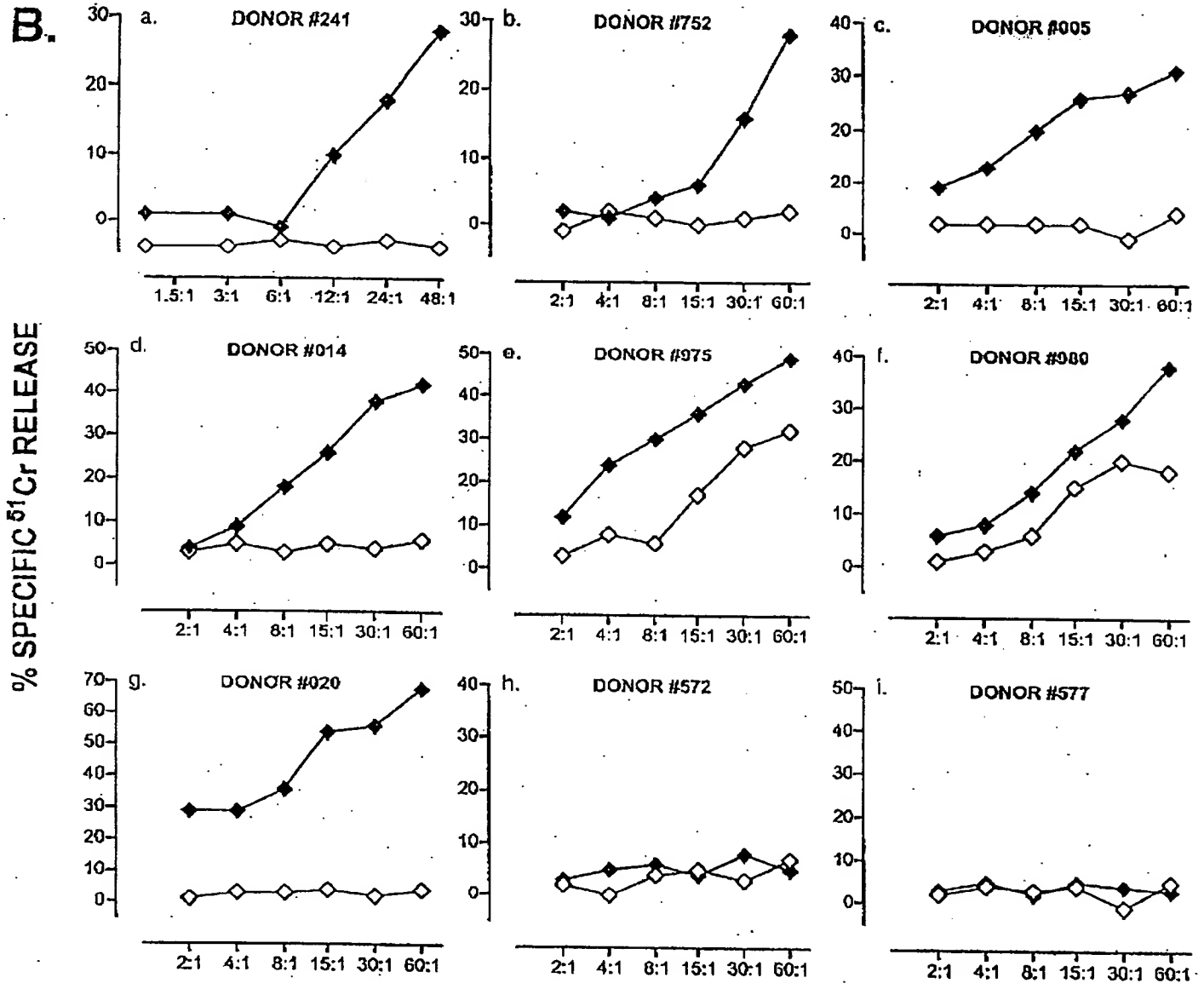
% SPECIFIC 51Cr RELEASE
0.8:1 1.5:1 3:1 6:1 12:1 24:1
1:1 2:1 4:1 8:1 16:1 32:1
2:1 4:1 8:1 15:1 30:1 60:1
EFFECTOR TO TARGET RATIO

B.


a. DONOR #241
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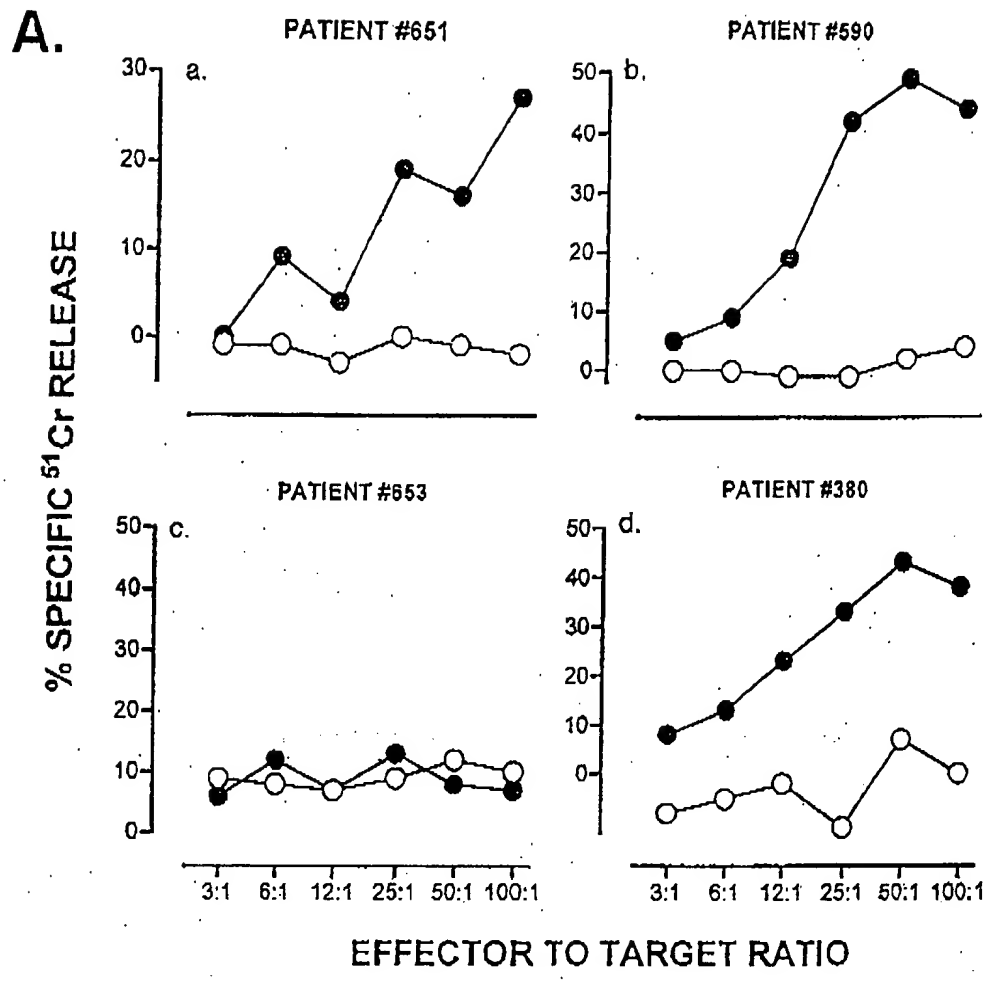
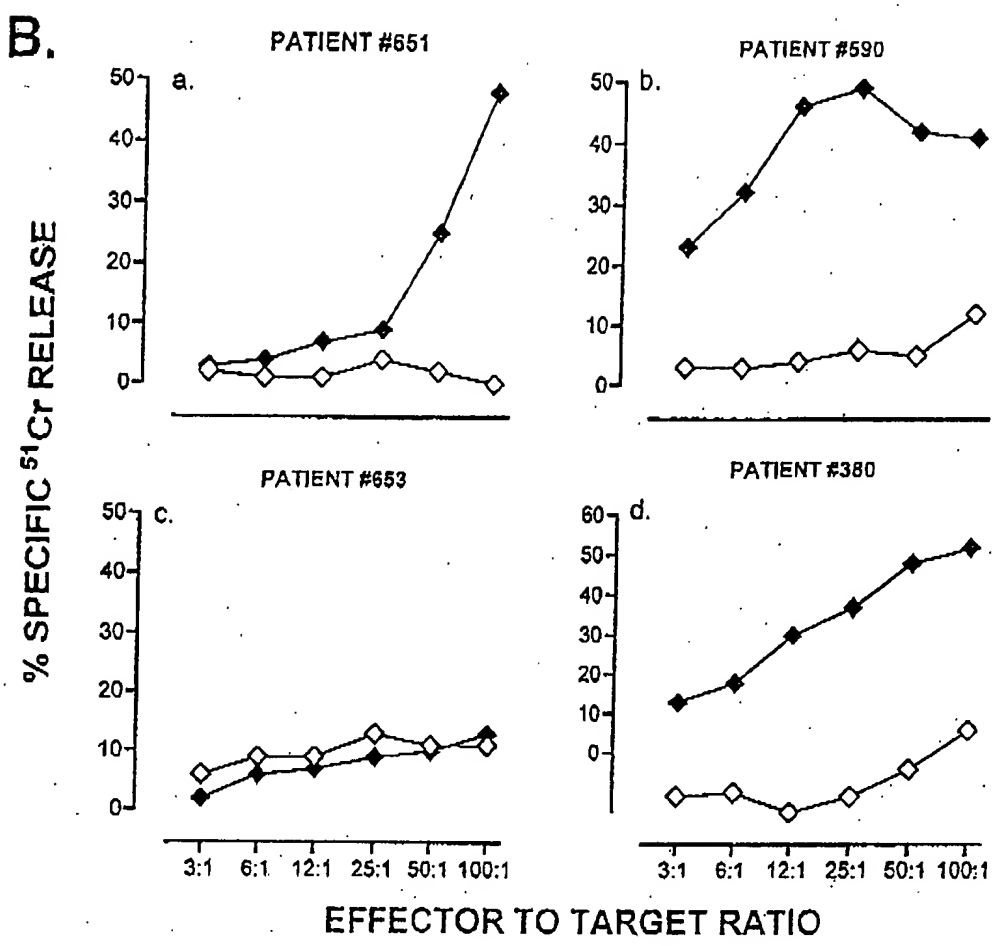
d. DONOR #014
e. DONOR #975
f. DONOR #980
g. DONOR #020
h. DONOR #572
i. DONOR #577
1.5:1 3:1 6:1 12:1 24:1 48:1
2:1 4:1 8:1 15:1 30:1 60:1
% SPECIFIC 51Cr RELEASE
EFFECTOR TO TARGET RATIO

Explanation to Exhibit 5. Induction of CTL against hTRT in PBMC from normal blood donors. T cells from HLA-A2 $^{+}$ individuals were stimulated by autologous PBMC pulsed with hTRT-derived synthetic peptides. (A). Results refer to effector cells from individual donors immunized *in vitro* against p540. Open circles define T2 cells and closed circles T2 cells pulsed with p540 as targets. (B). Results refer to effector cells from individual donors immunized *in vitro* against p865. Open diamonds define T2 cells and closed diamonds T2 cells pulsed with p865 as targets. Effector to target ratios are indicated on an individual basis.

EXHIBIT 6


A.
PATIENT #651
a.
PATIENT #590
b.
PATIENT #653
c.
PATIENT #380
d.
% SPECIFIC 51Cr RELEASE
30
20
10
0
50
40
30
20
10
0
3:1
6:1
12:1
25:1
50:1
100:1
EFFECTOR TO TARGET RATIO


B.
PATIENT #651
a.
PATIENT #590
b.
PATIENT #653
c.
PATIENT #380
d.
% SPECIFIC 51Cr RELEASE
3:1 6:1 12:1 25:1 50:1 100:1
EFFECTOR TO TARGET RATIO

Explanation to Exhibit 6. Induction of CTL against hTRT in PBMC from prostate cancer patients. (A). Results refer to effector cells from individual patients immunized against p540. Values refer to cells tested after three rounds of *in vitro* stimulation. Open circles define T2 cells and closed circles T2 cells pulsed with p540 as targets. (B). Results refer to effector cells from individual patients immunized against p865. Open diamonds define T2 cells and closed diamonds T2 cells pulsed with p865 as targets. Effector to target ratios are indicated on an individual basis.